

Application No. 10/735,601  
Filed December 12, 2003  
Response to Office Action Dated February 9, 2007  
Amendment Filed August 8, 2007

**The following Listing of the Claims replaces all prior Listings of the Claims.**

Please amend the claims as follows:

1. (Withdrawn) A method for preparing alphaviral replicon particles (ARPs) encoding and expressing a plurality of antigens, said method comprising the steps of:
  - a) introducing a plurality of alphaviral replicon nucleic acids into a plurality of cells, wherein said cells are permissive for alphavirus replication and packaging, wherein said replicon nucleic acid comprises at least a virus packaging signal and at least one heterologous coding sequence expressible in said alphaviral replicon nucleic acid, wherein said cell comprises at least one helper function, to produce a modified cell, and wherein the plurality of alphaviral replicon nucleic acids encode a plurality of antigens, to produce a plurality of modified cells;
  - b) culturing said plurality of modified cells of step (a) under conditions allowing expression of the at least one helper function, allowing replication of said alphaviral replicon nucleic acid and packaging of said alphaviral replicon nucleic acid to form ARPs;
  - c) contacting the modified cells after step (b) with an aqueous solution having an ionic strength from 0.2M to 5M to release the ARPs into the aqueous solution to produce a ARP-containing solution; and
  - d) collecting ARPs from the ARP-containing solution of step (c).
2. (Withdrawn) The method of claim 1 wherein the at least one helper function in the host cell of step (a) is encoded by a nucleic acid sequence stably integrated within the genome of said host cell.
3. (Withdrawn) The method of claim 1 wherein the at least one helper function in the cell is introduced on at least one helper nucleic acid which

- encodes a capsid protein capable of binding said alphaviral replicon nucleic acid, and at least one alphaviral glycoprotein, wherein said alphaviral glycoprotein associates with said alphaviral replicon nucleic acid and said capsid protein, wherein the at least one helper nucleic acid molecule is introduced into the cell together with said alphaviral replicon nucleic acid.
4. (Withdrawn) The method of claim 1, wherein the at least one helper function is encoded by at least two helper nucleic acid molecules wherein each of said two helper nucleic acid molecules encodes at least one alphaviral helper function.
  5. (Withdrawn) The method of claim 1, wherein the at least one helper nucleic acid molecule and the alphaviral replicon RNA are RNA molecules.
  6. (Withdrawn) The method of claim 5, wherein the at least one helper nucleic acid molecule is not capped.
  7. (Withdrawn) The method of claim 1, wherein at least one helper nucleic acid molecule is a DNA molecule.
  8. (Withdrawn) The method of claim 1, wherein the replicon nucleic acid is introduced into said host cell by electroporation.
  9. (Withdrawn) The method of claim 8, wherein the cell density in the electroporation milieu is from  $10^7$  to  $5 \times 10^8$  per mL.
  10. (Withdrawn) The method of claim 8, wherein the electroporation is carried out in an electroporation cuvette.
  11. (Withdrawn) The method of claim 1, wherein step (d) is followed by an ion exchange chromatography step or a heparin affinity chromatography step.

12. (Withdrawn) The method of claim 1, wherein the alphavirus is an attenuated alphavirus.
13. (Withdrawn) The method of claim 12, wherein the attenuated alphavirus is Venezuelan equine encephalitis virus (VEE).
14. (Withdrawn) The method of claim 13, wherein the attenuated VEE is strain 3014.
15. (Withdrawn) The method of claim 1, wherein the wash step employs NaCl, KCl, MgCl<sub>2</sub>, CaCl<sub>2</sub>, NH<sub>4</sub>Cl, (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, NH<sub>4</sub> Acetate or NH<sub>4</sub> Bicarbonate.
16. (Currently amended) An alphavirus replicon particle preparation prepared by ~~the method of claim 1~~
  - a) introducing a plurality of alphaviral replicon nucleic acids into a plurality of cells, wherein said cells are permissive for alphavirus replication and packaging, wherein said replicon nucleic acid comprises at least a virus packaging signal and at least one heterologous coding sequence expressible in said alphaviral replicon nucleic acid, wherein said cell comprises at least one helper function, to produce a modified cell, and wherein the plurality of alphaviral replicon nucleic acids encode a plurality of antigens corresponding to a nucleic acid expression library synthesized from an antigen source, to produce a plurality of modified cells;
  - b) culturing said plurality of modified cells of step (a) under conditions allowing expression of the at least one helper function, allowing replication of said alphaviral replicon nucleic acid and packaging of said alphaviral replicon nucleic acid to form ARPs, wherein said ARPs bind to heparin;
  - c) contacting the modified cells after step (b) with an aqueous solution having an ionic strength from 0.2M to 5M to release the ARPs into the aqueous solution to produce a ARP-containing solution; and

- d) collecting ARPs from the ARP-containing solution of step (c),  
whereby an alphavirus replicon particle preparation which encodes an expression library is produced.
17. (Currently amended) The alphavirus replicon particle preparation of claim 16, wherein the plurality of encoded antigens are derived from antigen source is tumor cells.
18. (Currently amended) The alphavirus replicon particle preparation of claim 16, wherein the plurality of encoded antigens are derived from antigen source is a parasite or a pathogen.
19. (Currently amended) The alphavirus replicon particle preparation of claim 18, wherein the plurality of encoded antigens are derived from antigen source is a pathogen selected from the group consisting of viruses, fungi, yeasts, bacteria and protozoans.
20. (Withdrawn, currently amended) A method for immunizing a human or animal against a parasite, pathogen or cancer, said method comprising the step of administering an amount of a virus replicon particle preparation of claim 15 16, effective for generating an immune response to at least one antigen of said parasite, pathogen or cancer.
21. (Withdrawn) The method of claim 20, wherein the pathogen is a virus, a bacterium, a yeast, a fungus or a protozoan.
22. (Withdrawn) The method of claim 21, wherein the virus is an influenza virus, a herpes virus, a parainfluenza virus, respiratory syncytial virus, cytomegalovirus, human papilloma, or human immunodeficiency virus.

23. (Withdrawn) The method of claim 21, wherein the protozoan is *Plasmodium falciparum*.
24. (Withdrawn) The method of claim 21, wherein the bacterium is *Mycobacterium tuberculosis*.
25. (Withdrawn) The method of claim 20, wherein the cancer is selected from the group consisting of pancreatic cancer, kidney cancer, sarcoma, neuroblastoma, glioma, colon cancer, melanoma, breast cancer, ovarian cancer and prostate cancer.
26. (Withdrawn) A method for preparing alphaviral replicon particles (ARPs) encoding and expressing a plurality of antigens, said method comprising the steps of:
  - a) introducing a plurality of alphaviral replicon nucleic acids into a plurality of cells, wherein said cells are permissive for alphavirus replication and packaging, wherein said replicon nucleic acid comprises at least a virus packaging signal and at least one heterologous coding sequence expressible in said alphaviral replicon nucleic acid, wherein said cell comprises at least one helper function, to produce a modified cell, and wherein the plurality of alphaviral replicon nucleic acids encode a plurality of antigens, to produce a plurality of modified cells, wherein the step of introducing the nucleic acids is by electroporating said cells at a density from  $5 \times 10^7$  to  $5 \times 10^8$  per mL of electroporation mixture;
  - b) culturing said plurality of modified cells of step (a) under conditions allowing expression of the at least one helper function, allowing replication of said alphaviral replicon nucleic acid and packaging of said alphaviral replicon nucleic acid to form ARPs;

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- c) contacting the modified cells after step (b) with an aqueous solution having an ionic strength from 0.2M to 5M to release the ARPs into the aqueous solution to produce a ARP-containing solution; and
  - d) collecting ARPs from the ARP-containing solution of step (c).
27. (Withdrawn) The method of claim 1 wherein the plurality of antigens is derived from a virus, a bacterium, a yeast, a fungus, a protozoan or a cancer cell.
28. (Withdrawn) The method of claim 27, wherein the virus is an influenza virus, a herpes virus, a parainfluenza virus, respiratory syncytial virus, cytomegalovirus, human papilloma, or human immunodeficiency virus.
29. (Withdrawn) The method of claim 27, wherein the protozoan is *Plasmodium falciparum*.
30. (Withdrawn) The method of claim 27, wherein the bacterium is *Mycobacterium tuberculosis*.
31. (Withdrawn) The method of claim 27, wherein the cancer cell is selected from the group consisting of a pancreatic cancer cell, a kidney cancer cell, sarcoma cell, neuroblastoma cell, glioma cell, colon cancer cell, melanoma cell, breast cancer cell, ovarian cancer cell and prostate cancer cell.